Reduction of fertilizing capacity of epididymal spermatozoa by 5α-steroid reductase inhibitors

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Summary. Antifertility effects on epididymal spermatozoa were tested by administration of 5α-reductase inhibitors to androgen substituted adult castrated male mice. The inhibitors depressed the in vitro fertilizing ability and in vitro blastocyst development in testosterone substituted castrated male mice.

It has been demonstrated that in the epididymides of various species the maturation and storage of spermatozoa are androgen-dependent. After castration, the fertilizing capacity of epididymal spermatozoa can be maintained when testosterone (T), dihydrotestosterone (DHT) or 3α-androstanediol (3α-diol) are administered[2]. Although the conversion from T to DHT is rather extensive and DHT is the major androgen in the nuclei of the epididymal cells[3], the conversion from T into DHT has never been proved to be essential for normal epididymal function. It has been reported that the concept of DHT as the growth promoting active androgen in most target organs can be demonstrated by the use of 4-androsten-3-one-17β-carboxylic acid (17βC). Growth of accessory sex tissues of T substituted castrated mice was reduced by 17βC treatment, but not by 17βC administered in combination with DHT[4]. Different in vitro studies have shown that 17βC is a potent inhibitor of T 5α-reductase[5-7]. In the present study we have investigated the effects of the 5α-reductase inhibitor 17βC and its methyl ester (17βME) on the maintenance of the fertilizing capacity of epididymal spermatozoa in castrated mice injected with T. Besides 17βC, 17βCME was injected since it can be argued that this anti-androgenic compound[5] might have a prolonged action in comparison to 17βC.

Materials and methods. Young adult male (C57BLxCBA)-F1 mice were castrated under tribromoethanol anaesthesia (each animal 10 mg). Starting on the day of castration the animals received daily s.c. injections of different steroids (Steraloids, Pawling, N.Y.), dissolved in 0.05 ml of sesame oil as outlined in the table. The concentrations administered were 5 μg testosterone propionate (TP), 10 μg dihydrotestosterone propionate (DHTP), 100 μg 17βC and 100 μg 17βME. On day 11, 17 h after the last injection, the animals were killed by cervical dislocation. One cauda epididymidis and ductus deferens were removed and placed in a sterile petri dish (Falcon) in 2 ml of an embryo culture medium[8]. After cutting the tissues into pieces the petri dishes were placed in an incubator at 37 °C and gassed with 5% CO_2 in air, allowing the spermatozoa to disperse into the medium. Each sperm suspension was tested with eggs of 2 superovulated (PMSG and hCG) 8-12-week-old female (C57BLxCBA)-F1 mice. Recently ovulated oocytes were isolated from the oviducts 20 h after the hCG injection and released into 0.2 ml of medium under liquid paraffin oil (Baker). To start in vitro fertilization, a drop of 0.2 ml from the sperm suspensions was added to the cumulus masses. After 7 h oocytes were removed, washed 3 times, and checked for the presence of 2 pronuclei. For an additional 96 h the embryos were cultured in Whitten's medium to the blastocyst stage.

Results. Both the total numbers of spermatozoa and the numbers of motile spermatozoa present in the epididymides of mice treated with TP, DHTP or DHTP and inhibitors were approximately half the numbers found in intact mice. Although a significant difference between the sperm numbers of group 2 versus groups 3 and 4 was found, no significant differences either in sperm number or in motility between groups 1 and 2 were obtained. In our assay we found that sperm suspensions from intact mice only fertilize a lower percentage of eggs when the sperm concentration diminishes to below 0.5×10^6 spermatozoa/ml. However, the incubated sperm suspensions obtained from the epididymides of the first 4 groups and of group 7 in the present experiment ranged from 0.9 to 6.0×10^6 spermatozoa/ml. From 202 fertilized eggs of intact controls 145 developed into blastocysts. Oocytes fertilized by spermatozoa from androgen substituted animals developed more blastocysts then oocytes fertilized by spermatozoa from animals treated with TP and inhibitors. Since the quantities of TP and DHTP administered were just sufficient to maintain the epididymal function, an effect of 5α-reductase inhibition was easily detectable. Animals treated with TP and DHTP (groups 1, 3 and 4) were all fertile, whereas 4 out of 6 animals injected with TP and inhibitors (group 2) were fertile. The fertilization percentage of group 2 was significantly lower than those of groups 1, 3 and 4, which shows that this is a direct effect of 17βC and 17βME.

Discussion. According to our results 17βC and 17βME inhibit the fertilizing capacity of epididymal spermatozoa in T substituted castrated mice. Effects of the inhibitors on the in vitro fertilization rate of epididymal spermatozoa

Effects of administration of 17βC and 17βME on the in vitro fertilizing capacity of epididymal spermatozoa, the motility and the number of spermatozoa in one epididymis

Group	Daily injections with	No. of fertile/ total number of mice	Percentage of fertilized oocytes	Total number of spermatozoa ($\times 10^6$/ml)	Number of motile spermatozoa ($\times 10^6$/ml)	Percentage blastocyst formation
1	TP	6/6	36± 5	2.5±0.4	1.5 ±0.3	52
2	TP+17βC+17βME	4/6	8± 4[a]	1.6±0.3[b]	0.9 ±0.2[c]	22[d]
3	DHTP	6/6	38±10	2.7±0.5	1.7 ±0.4	56
4	DHTP+17βC+17βME	6/6	41±11	2.9±0.4	1.4 ±0.2	66
5	17βC+17βME	1/5	6± 6	0.3±0.2	0.02±0.02	33
6	Vehicle only	0/6	0	0.2±0.2	0	-
7	Intact control	6/6	86± 5	5.7±0.4	3.6 ±0.4	72

Data for 6 different groups of castrated and androgen substituted male mice compared with noncastrated intact controls. Mean values±SEM. An overall analysis of variance was made and individual pairs of values were compared by LSD-tests. [a] Group 2 versus groups 1, 3 and 4; $p < 0.05$; [b] group 2 versus groups 3 and 4; $p < 0.05$; [c] group 2 versus group 3; $p < 0.05$; [d] group 2 versus groups 1, 3 and 4; $p < 0.05$.

from castrated mice can be detected after 10 days of administration. Our results extend the data published by Lau, Saksena and Chang[9]. It was shown by these authors that 17βC administered s.c. to intact male mice during 15 days decreased the fertilizing ability of inseminated spermatozoa. However, in their study the reduction in fertility might also be caused by a direct or indirect influence of the 5α-reductase inhibitors on the testicular T secretion. Significant differences of sperm number and motility between animals substituted with TP only and animals substituted with TP and inhibitors were not observed in our study. This means that a decrease in spermatozoal fertility due to an inhibition of 5α-steroid reductase activity in the epididymis preceded a reduction in sperm number and motility. The effects on epididymal spermatozoa caused by administration of 17βC and 17βME are not only apparent from decreased fertilization percentages but also from decreased in vitro blastocyst formation. From results presented here it is indicated that the inhibitors studied are not toxic and exert a negligible androgenic action on epididymal tissue.

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Modification of pulsatile pattern of basal insulin secretion in the dog by general anesthesia (Nembutal)

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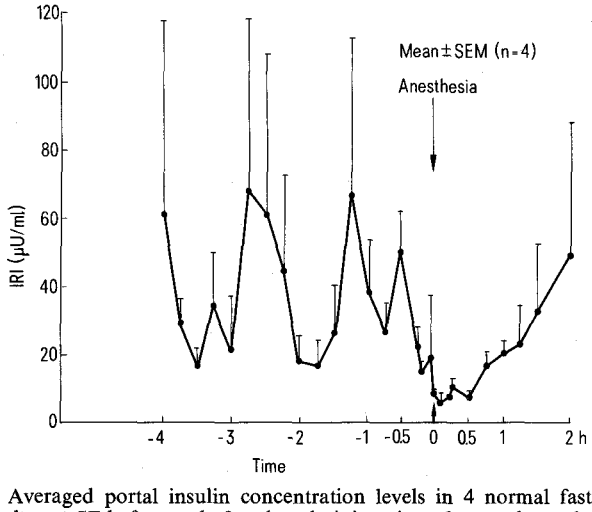
Summary. Pulsatile pattern of basal insulin secretion in conscious dog can be modified by the administration of general anesthesia (nembutal): the amplitude of secretory bursts is dramatically reduced. The possibility that a primary control of basal insulin secretion is in the CNS cannot be excluded.

It is generally accepted that the secretion of insulin is regulated primarily by substrate availability and it is the subject to neural and hormonal control.

However it was shown, for the first time, by Vanhelder et al.[1] that insulin is secreted in portal blood of conscious normal dogs in the form of intermittent bursts. These bursts which are not reflected in the periphery are substrate independent and on occasions exceed up to 20-fold the basal secretory insulin level. While the physiological significance of these surges has not been clarified yet, the fact that they can be manipulated by general anesthesia (nembutal) can bring some light into this phenomenon.



Averaged portal insulin concentration levels in 4 normal fasting dogs ± SE before and after the administration of general anesthesia (nembutal).

Materials and methods. 4 normal male mongrel dogs were used (weight between 13 and 20 kg). They were fitted with indwelling silastic portal and jugular catheters under general anesthesia (nembutal, 35 mg/kg) and aseptic conditions. The catheters were filled with heparinized saline (1:40). 2 weeks after the cathetarization, when the animals recovered, the experiment was performed. In these 2 weeks the animals were often handled and got used to withdrawal of blood samples. During the experiment blood samples were taken every 15 min for 7-12 h from the catheters. After each blood sample had been taken the blood was replaced by the same volume of saline. 2 h before the experiment was terminated the animals were anesthetized by nembutal (35 mg/kg). The stage of surgical anesthesia was reached and maintained. Samples continued to be taken in the anesthetized animals. Samples were withdrawn also at times -10, -3, $+2$, $+5$ and $+10$ min with respect to the administration of anesthesia. Plasma glucose was determined in fresh samples by the Beckman analyser (true glucose) and insulin was determined in stored, frozen samples, after thawing, by the double antibody assay of Hales and Randle[2] using the kit of Amersham Searle Corporation (Chicago, Ill.). Mean insulin levels ± SE values were calculated for 4 h before and 2 h after the administration of general anesthesia.